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Stereochemical Basis for the Insecticidal Activity of Carbamoylated and Acylated Pyrazolines

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Abstract: Methyl 3-(4-chlorophenyl)-1-[*N*-(4-chlorophenyl)carbamoyl]-4-methyl-2-pyrazoline-4-carboxylate was converted to corresponding (1*R*)- and (1*S*)-phenethyl esters via its carboxylic acid and acid chloride at the C-4 atom to separate the diastereomers. Their configurations were confirmed by X-ray analysis. Both isomers of the (1*R*)methylbenzyl ester were subjected to transesterification with sodium methoxide to obtain enantiomers of the starting methyl ester. Their insecticidal activity was measured against American cockroaches (*Periplaneta americana* (L.)) by injection and against house flies (*Musca domestica* L.) by topical application under various synergistic conditions with metabolic inhibitors. The activity values of the four α-methylbenzyl esters and the *R*-isomer of the starting methyl ester were similar. The *S*-enantiomer of the methyl ester was about 10 and 100 times more active than the *R*-isomer against the cockroach and the fly, respectively. Some *N*-arylacetyl and *N*-aryloxyacetyl derivatives of the starting *N*-(4-chlorophenyl)carbamoyl compound gave very low activity. Conformation-energy profiles for some compounds suggested that the conformation of substituents on the N-1 atom in the pyrazoline ring has a specific role for the potential insecticidal effects.

Key words: stereochemistry, enantiomers, insecticide, pyrazoline, American cockroach, house fly.

1 INTRODUCTION

The *N*-phenylcarbamoylpyrazolines have potent insecticidal activity against a broad spectrum of insects.[1,2] At an early stage of structural modification, the 4-position of the pyrazoline ring was unsubstituted or monosubstituted.[2,3] However, these compounds had undesirable characteristics as insecticides, especially instability due to photoaromatization and a long half-life in soil.[4,5] After structural modification,[6] some compounds including compound **2** (Fig. 1; A = CH_3, B = $COOCH_3$, X = Y = 4-Cl, Z = O), which have two substituents at the 4-position, were found to have a short half-life in soil and were less affected by photoaromatization.[7]

There are many examples to show different levels of biological potency between enantiomers. Compounds of structure **A** (Fig. 1) have a chiral center, so that elucidation of their chiral effects was considered, to give more information on structure–activity relationships for this class of insecticide. A racemic mixture of one of these compounds (A = 4-F-C_6H_4, B = H, X = 4-

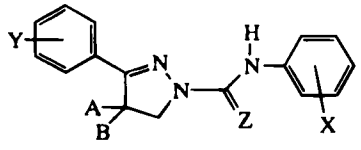

Fig. 1. General structure, **A**, of compounds discussed.

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$OCHF_2$, Y = 4-OCH_2CF_3, Z = O) has been separated through an optically active camphanic acid derivative, but no biological data were reported.[8] Even though some compounds having the structure **A** have been shown to modulate nerve functions related to sodium[9,10] and calcium channels,[11] the precise site of action remains to be elucidated. Thus, enantiomers of this class of compounds may also be excellent tools to study the critical site of action for the insecticidal effect.

Variations in the insecticidal activity of a set of compounds of structure **A** (A = CH_3, B = $COOCH_3$, Y = 4-Cl, Z = O) have been analysed quantitatively by using physicochemical substituent parameters.[12] One of the findings was that the more the electron-withdrawing property of the *para* substituents, the higher was the activity. It was suggested that the electron density around the NH group of the (*para*-substituted phenyl)carbamoyl moiety has some critical role in enhancing the insecticidal activity.

Recently, we have prepared four stereoisomers related to compound **2** by introducing optically defined alcohols in place of the methyl ester moiety of the side chain. From the stereochemically defined isomers, enantiomers of compound **2** were derived. In the present paper it is shown that the *S*-isomer is about 10 times more insecticidally active than the *R*-isomer against American cockroaches when they are injected. Similar results were obtained against house flies. It is also shown that the insecticidal activity of the analogue of compound **2** having a 4-chlorophenylacetyl group on the N-1 atom of the pyrazoline ring was very low. These findings will be discussed in connection with the energy barriers for rotation around the C(=O)—NH bond of compound **2** and the C(=O)—CH_2 bond of the phenylacetyl derivative.

2 MATERIALS AND METHODS

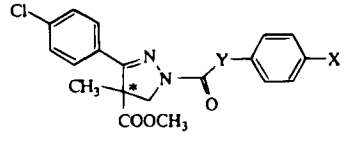
2.1 Compounds

Except for compounds **1** and **2** that were reported in a previous paper,[12] compounds listed in Tables 1 and 2 were prepared according to the methods outlined in Fig. 2.[6,12] The yields described below were not optimized. The [^{1}H]NMR spectra were measured in deuterochloroform, using tetramethylsilane as an internal standard, with JEOL PMX (60 MHz) and Bruker AC-300 (300 MHz) instruments. The optical rotation was measured with a JASCO ORD model J-5 spectropolarimeter. The compounds were confirmed either by high-resolution electron-impact mass spectrometry (HR-EI-MS; JEOL JMS-HX/HX110A) measured at the Faculty of Pharmacy or by elementary analysis for C, H and N at the Elementary Analysis Center of this university. The absolute configuration of one of the stereoisomers of the 1-methylbenzyl ester, the preparation of which will be described below, was confirmed by X-ray analysis with Rigaku AFC-5 refractometer. The melting points were measured with a Yanagimoto micromelting point apparatus and were uncorrected.

NIA 16388 (propargyl propyl benzenephosphonate; NIA), an inhibitor of hydrolytic metabolism, was the same sample used in work reported previously.[12]

TABLE 1

Insecticidal Activity of Pyrazolines against American Cockroaches and House Flies under Various Synergistic Conditions



Compound	X	Y	Configuration	log(1/MLD) (cockroach)			log(1/LD_{50}) (fly)[a]	
				Alone	PB	PB + NIA	Alone	PB + NIA
1	H	NH	*RS*	6·99	7·69	8·06	< 7·80 (40%)	8·32
2	Cl	NH	*RS*	8·74	9·00	9·34	9·50	9·76
2a	Cl	NH	*R*	7·97	8·67	8·76	7·78	8·05
2b	Cl	NH	*S*	9·19	9·40	9·70	9·75	10·08
3	H	CH_2	*RS*	—	—	6·85	—	< 8·31 (3%)
4	Cl	CH_2	*RS*	—	—	6·86	—	< 8·14 (8%)
5	H	CH_2O[b]	*RS*	—	—	6·84	—	< 8·09 (3%)
6	Cl	CH_2O[b]	*RS*	—	—	6·96	—	< 8·14 (5%)

[a] Percentage in parentheses indicates that of killed insects at the stated dose.
[b] An aryl ether.

TABLE 2
Insecticidal Activity of Stereoisomers of 1-Methylbenzyl 3-(4-Chlorophenyl)-1-[*N*-(4-chlorophenyl)carbamoyl]-4-methyl-2-pyrazoline-4-carboxylate

	Configurations		*log(1/MLD)*		
Compound	*Position 4*	*Position 1'*	*Alone*	*PB*	*PB + NIA*
7a	*R*	*R*	7·51	7·51	8·50
7b	*S*	*R*	7·51	7·51	8·50
8a	*R*	*S*	7·52	7·92	8·61
8b	*S*	*S*	7·43	7·73	8·53

Reagent grade piperonyl butoxide (PB) was used as an inhibitor of oxidative metabolism. Metabolic inhibitors (synergists) were dissolved in methanol.

2.1.1 Methyl (4RS)-3-(4-chlorophenyl)-4-methyl-1-phenylacetyl-2-pyrazoline-4-carboxylate (II, X = H, Y = CH_2 ; compound 3)

To a solution of (4*RS*)-3-(4-chlorophenyl)-4-methoxycarbonyl-4-methyl-2-pyrazolinium chloride (Fig. 2, **I**; 0·50 g, 1·7 mmol) (which was prepared according to the reported method)[6,12] stirred at room temperature in dichloromethane (10 ml) was added triethylamine (0·86 g, 8·5 mmol) in dichloromethane (5 ml) and then phenylacetyl chloride (0·39 g, 2·6 mmol) in dry benzene (5 ml). After stirring overnight, the reaction mixture was diluted with dichloromethane. The mixture was washed consecutively with hydrochloric acid (3 M), saturated sodium hydrogen carbonate, and saturated sodium chloride and the organic layer was dried over magnesium sulfate. After concentration, the residue was chromatographed over silica gel with hexane + ethyl acetate (4 + 1 by volume) as an eluent. The solid obtained from the major fraction was further purified by recrystallization from a mixture of hexane and ethyl acetate. Yield, 0·20 g (32%), m.p. 107–108°C. [^{1}H]NMR δ ppm: 7·70–7·20 (9H, m, aromatic protons), 4·41 (1H, d, J = 12·0 Hz, CH_2), 4·09 (2H, s, CH_2-C_6H_5), 4·08 (1H, d, J = 12·0 Hz, CH_2), 3·68 (3H, s, OCH_3), 1·59 (3H, s, C-CH_3).

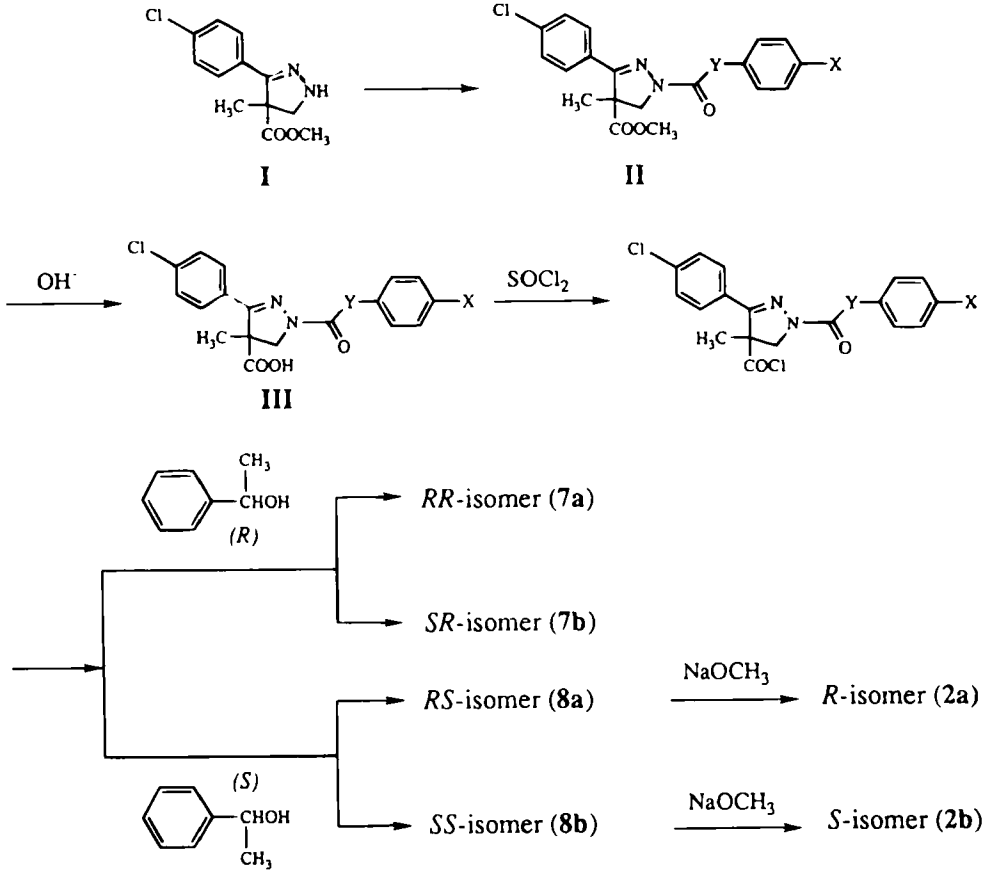


Fig. 2. Synthetic scheme for compounds listed in Tables 1 and 2.

Compounds **4–6** were prepared similarly. M.p. 121–122°C (compound **4**), 112–113°C (compound **5**), and 121–122°C (compound **6**).

2.1.2 3-(4-Chlorophenyl)-1-[N-(4-chlorophenyl)-carbamoyl]-4-methyl-2-pyrazoline-4-carboxylic acid (III, X = Cl, Y = NH)

Compound **2** (Fig. 2, **II**; X = Cl, Y = NH; 8·0 g, 0·02 mol), prepared according to the reported method,[6,12] was dissolved in a mixture of ethanol (20 ml) and sodium hydroxide (2 M; 60 ml) and kept at 90°C for 30 min.[13] The solution was cooled to room temperature and acidified to pH 2 with concentrated hydrochloric acid. The precipitated solid was collected and dried to afford compound **III** (X = Cl, Y = NH). Yield, 7·9 g, m.p. 200°C. [^{1}H]NMR δ ppm: 8·81 (1H, s, NH), 7·80–7·21 (8H, m, aromatic protons), 4·43 and 3·91 (2H, d, J = 11·0 Hz, CH_2), 1·61 (3H, s, CH_3). This product was used for the following reactions without further purification.

2.1.3 (1R)Methylbenzyl (4R)-3-(4-chlorophenyl)-1-[N-(4-chlorophenyl)carbamoyl]-4-methyl-2-pyrazoline-4-carboxylate (7a) and its (4S)-isomer (7b)

The carboxylic acid **III** (2·5 g; 6·22 mmol) was suspended in thionyl chloride (20 ml; 0·27 mol) and heated under reflux for 5 h. The excess thionyl chloride was evaporated and the residue dissolved in dichloromethane (15 ml). To the solution, pyridine (1·48 g; 18·7 mmol) in dichloromethane (5 ml) was added dropwise at 0°C. After 30 min, $R(+)$-α-phenethyl alcohol (1·0 g; 8·2 mmol) in dichloromethane (5 ml) was added dropwise and the reaction mixture was stirred for 5 h at room temperature. The reaction mixture was diluted with dichloromethane, washed with hydrochloric acid (1 M), saturated sodium hydrogen carbonate and sodium chloride solution, and the organic layer dried over magnesium sulfate. After concentration, the residue was chromatographed over silica gel, eluting with toluene + ethyl acetate (6 + 1 by volume) to obtain two fractions. Each of the fractions was rechromatographed over silica gel, eluting with hexane + ethyl acetate (8 + 1 by volume). After recrystallization from ethyl acetate and hexane, compounds **7a** (460 mg, 12·5%, m.p. 115–117°C, $[\alpha]_D^{25} = +113{\cdot}0°$ conc. 2% in methanol) and **7b** (374 mg, 10%, m.p. 102–103°C, $[\alpha]_D^{25} = -108{\cdot}0°$ conc. 2% in methanol) were obtained. [^{1}H]NMR, **7a**, δ ppm: 7·98 (1H, s, NH), 7·57 (2H, d, J = 8·8 Hz, aromatic protons), 7·49 (2H, d, J = 8·9 Hz, aromatic protons), 7·31 (2H, d, J = 8·8 Hz, aromatic protons), 7·29 (2H, d, J = 8·9 Hz, aromatic protons), 7·35–7·25 (5H, m, aromatic protons), 5·94 (1H, q, J = 6·6 Hz, $CHCH_3$), 4·33 and 3·98 (2H, d, J = 11·2 Hz, CH_2), 1·62 (3H, s, CH_3), 1·40 (3H, d, J = 6·6 Hz, $CHCH_3$). [^{1}H]NMR, **7b**, δ ppm: 7·99 (1H, s, NH), 7·51 (2H, d, J = 8·9 Hz, aromatic protons), 7·39 (2H, d, J = 8·9 Hz, aromatic protons), 7·29 (2H, d, J = 8·9 Hz, aromatic protons), 7·14 (2H, d, J = 8·9 Hz, aromatic protons), 7·31–7·02 (5H, m, aromatic protons), 5·92 (1H, q, J = 6·7 Hz, $CHCH_3$), 4·43 and 4·04 (2H, d, J = 11·1 Hz, CH_2), 1·60 (3H, s, CH_3), 1·52 (3H, d, J = 6·7 Hz, $CHCH_3$).

$C_{26}H_{23}O_3N_3Cl_2$:

Calcd C, 62·90; H, 4·68; N, 8·46 (%)

Found C, 63·15; H, 4·56; N, 8·53 (%) **(7a)**

Found C, 63·04; H, 4·72; N, 8·51 (%) **(7b)**

2.1.4 (1S)Methylbenzyl (4R)-3-(4-chlorophenyl)-1-[N-(4-chlorophenyl)carbamoyl]-4-methyl-2-pyrazoline-4-carboxylate (8a) and its (4S)-isomer (8b)

The carboxylic acid **III** (3·0 g; 7·46 mmol) was converted to the acid chloride, followed by treatment with $S(-)$-α-phenethyl alcohol (1·0 g; 8·2 mmol) by a method similar to that described above. The reaction mixture was worked up as described above to obtain two fractions corresponding to lower (**8a**, 383 mg, 10·5%, m.p. 102–103°C, $[\alpha]_D^{25} = -107{\cdot}0°$ conc. 2% in methanol) and upper (**8b**; 542 mg, 14·7%, m.p. 115–117°C, $[\alpha]_D^{25} = -112{\cdot}2°$ conc. 2% in methanol) spots on silica gel TLC. [^{1}H]NMR, **8a**, δ ppm: 7·99 (1H, s, NH), 7·50 (2H, d, J = 8·8 Hz, aromatic protons), 7·39 (2H, d, J = 8·6 Hz, aromatic protons), 7·29 (2H, d, J = 8·8 Hz, aromatic protons), 7·14 (2H, d, J = 8·6 Hz, aromatic protons), 7·31–7·02 (5H, m, aromatic protons), 5·92 (1H, q, J = 6·7 Hz, $CHCH_3$), 4·43 and 4·04 (2H, d, J = 11·1 Hz, CH_2), 1·60 (3H, s, CH_3), 1·52 (3H, d, J = 6·7 Hz, $CHCH_3$). [^{1}H]NMR, **8b**, δ ppm: 7·98 (1H, s, NH), 7·57 (2H, d, J = 8·7 Hz, aromatic protons), 7·49 (2H, d, J = 8·9 Hz, aromatic protons), 7·31 (2H, d, J = 8·6 Hz, aromatic protons), 7·28 (2H, d, J = 8·8 Hz, aromatic protons), 7·36–7·25 (5H, m, aromatic protons), 5·94 (1H, q, J = 6·6 Hz, $CHCH_3$), 4·34 and 3·98 (2H, d, J = 11·2 Hz, CH_2), 1·62 (3H, s, CH_3), 1·41 (3H, d, J = 6·6 Hz, $CHCH_3$).

$C_{26}H_{23}O_3N_3Cl_2$:

Calcd C, 62·90; H, 4·68; N, 8·46 (%)

Found C, 63·10; H, 4·66; N, 8·48 (%) **(8a)**

Found C, 63·19; H, 4·66; N, 8·43 (%) **(8b)**

2.1.5 Methyl (4R)-3-(4-chlorophenyl)-1-[N-(4-chlorophenyl)carbamoyl]-4-methyl-2-pyrazoline-4-carboxylate (2a) and its (4S)-isomer (2b)

A solution of (1S)methylbenzyl (4R)-3-(4-chlorophenyl)-1-[N-(4-chlorophenyl)carbamoyl]-4-methyl-2-pyrazoline-4-carboxylate (**8a**; 270 mg; 0·54 mmol) in dry methanol (10 ml) was refluxed with sodium methoxide (4·0 mg; 0·07 mmol) for 4 h.[14] The reaction mixture was diluted

with water and extracted with dichloromethane. The organic layer was washed with hydrochloric acid (1 M) and dried over magnesium sulfate. After concentration, the residue was chromatographed over silica gel with hexane + ethyl acetate (8 + 1 by volume) as the eluting system to afford compound **2a** (200 mg, 90%, m.p. 108°C, $[\alpha]_D^{25} = +104{\cdot}5°$ conc. 0·42% in methanol). The molecular ion (M^+) peak was observed at *m/z* 405·064 by HR-EI-MS analysis and the molecular formula was determined to be $C_{19}H_{17}O_3N_3Cl_2$ (calcd 405·064). [^{1}H]NMR δ ppm: 7·97 (1H, s, NH), 7·61 (2H, d, J = 9·0 Hz, aromatic protons), 7·49 (2H, d, J = 9·0 Hz, aromatic protons), 7·38 (2H, d, J = 9·0 Hz, aromatic protons), 7·29 (2H, d, J = 9·0 Hz, aromatic protons), 4·43 and 4·01 (2H, d, J = 11·0 Hz, CH_2), 3·76 (3H, s, OCH_3), 1·65 (3H, s, CH_3).

From (1*S*)methylbenzyl (4*S*)-3-(4-chlorophenyl)-1-[*N*-(4-chlorophenyl)carbamoyl]-4-methyl-2-pyrazoline-4-carboxylate (**8b**; 250 mg; 0·50 mmol), compound **2b** (90 mg, 45%, m.p. 114–117°C, $[\alpha]_D^{25} = -100{\cdot}0°$ conc. 0·42% in methanol) was prepared in a similar way. [^{1}H]NMR data were the same as those of compound **2a**. The molecular ion (M^+) peak was observed at *m/z* 405·065 by HR-EI-MS analysis and the molecular formula was determined to be $C_{19}H_{17}O_3N_3Cl_2$ (calcd 405·064).

2.2 Molecular modelling

All computations were done with the molecular modelling software package SYBYL, version 5.41.[15] To select initial conformation on the compounds, we started from the coordinates of X-ray crystallographic data for compound **8a**. The alcohol moiety of the compound was replaced by a methyl group to generate compound **2a**. The coordinates of the methyl group were calculated by use of the SYBYL standard values for bond lengths and angles,[15] with the structures of the rest of the molecule left unattached. Coordinates obtained after manipulation of the X-ray structure for the compound were fully optimized by the semi-empirical molecular orbital method, PM3.[16] The C(=O)—NH bond of the carbamoyl moiety was first rotated so as to give the *anti* conformation regarding the N^1—C(=O) and NH—C_6H_4(*p*—Cl) bonds, the torsion angle of which was defined as 180°, and was rotated from 0° to 360° in 15° increments to calculate the relative heats of formation (kcal mol^{-1}) for each conformer by PM3. The NH group of compound **2a** with the *anti* conformation was substituted by CH_2 to give the *anti* conformer of the *R*-isomer of compound **4** by using the SYBYL standard values for bond lengths. As for compound **2a**, the C(=O)—CH_2 bond of the N^1-substituent was rotated from 0° to 360° in 15° increments to calculate the energy for each conformer. The energy for each conformer for each compound was expressed as a value relative to that of the conformer having the minimum energy.

2.3 Insecticidal tests

2.3.1 American cockroaches

The insecticidal activity of the compounds was measured against one- to three-month-old male adult American cockroaches, *Periplaneta americana* (L.), by the procedure described previously.[12] Various volumes (1–10 μl) of the methanol solution of each compound were injected into the abdomen of the insects so as to make the doses at 0·1 intervals in log units. In some experiments, a methanol solution (1 μl) containing PB (50 μg) or PB (50 μg) plus NIA (50 μg) was injected into the abdomen 1 h before the injection of the test compounds to suppress the metabolic mechanism. Three insects were used to test each dose of each compound. The insects were kept at 20(±1)°C for 24 h. When the dose was sufficient, the insects fell over and were unable to retain the right posture, leading to paralysis that was usually associated with violent tremors. The minimum dose at which two out of three insects died or were paralyzed was considered as the minimum lethal dose (MLD in moles). The volume of injected methanol solution (1–10 μl) with or without synergists did not affect the MLD value. For each series of experiments, at least 25 insects were used. Measurement of MLD was repeated until values were found to be reproducible. The log(1/MLD) values for test compounds are listed in Tables 1 and 2. Each value is the mean for at least two repetitions with a standard error of ±0·2.

2.3.2 House flies

Adult females, four to seven days old, of a strain of susceptible house flies (*Musca domestica* L., Takatsuki) were used in the test. The activity of the compounds was measured by a procedure described previously.[17] A methanol solution (1 μl) containing various amounts of compounds was topically applied to the ventral side of the abdomen. To suppress the metabolic mechanism, 1 μl of the methanol solution containing PB (2 g litre^{-1}) plus NIA (2 g litre^{-1}) was applied to the abdomen immediately before the topical application of the insecticides. Methanol alone in this volume and synergists alone in these amounts caused no toxic effect on the flies. The lethal effect, defined as the dropping of the insects to the bottom of the container due to convulsion and/or paralysis, was monitored at intervals at 24(±1)°C. About 100 insects were used for each dose of each compound. Whichever synergistic or nonsynergistic condition was used, the increase in the number of affected insects ceased 8–10 h after application of the insecticides as exemplified in Fig. 3 for compound **2**. Rate of development of the killing effect seemed to be little affected by the synergists. The dose–response

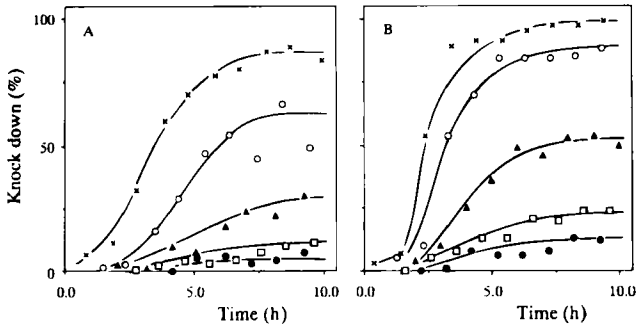


Fig. 3. Time–response relationship for effect of **2** against house flies. The compound (●) $4{\cdot}84 \times 10^{-11}$ mol; (□) $9{\cdot}68 \times 10^{-11}$ mol; (▲) $1{\cdot}94 \times 10^{-10}$ mol; (○)$3{\cdot}87 \times 10^{-10}$ mol; (×) $7{\cdot}75 \times 10^{-10}$ mol was topically applied (A) without and (B) with PB (2 μg) and NIA (2 μg).

relationship was therefore examined from data at 10 h after the application. From such a relationship, LD_{50}, the dose (in moles) with which 50% of the insects were killed, was calculated by probit transformation. The $\log(1/LD_{50})$ values are given in Table 1. The standard error of the potency was within ±0·2.

3 RESULTS

3.1 Chiral effects at the pyrazoline ring on the insecticidal activities against American cockroaches and house flies

Chiral effects at the C-4 atom of the pyrazoline ring on the insecticidal activities were examined for the racemic compound **2** and its enantiomers **2a** and **2b**. PB increased the activity of these compounds against American cockroaches by 0·2–0·7 in log units (Table 1). By additional use of NIA, their activity increased by 0·5–0·8 in log units as compared with that determined without the synergists. Without the synergists or with PB or PB plus NIA, the *S*-isomer **2b** was about 10 times more active than the *R*-isomer **2a**. Similar chiral effects were also observed against house flies. The unsubstituted compound **1** was weaker than the *para*-chloro derivative (compound **2**) against both insect species. The synergistic effects of a mixture of PB and NIA on the insecticidal activity of compounds **1** and **2** against house flies were slightly less than those against American cockroaches (Table 1). With and without PB and NIA, the insecticidal activity of the *S*-isomer **2b** against house flies was about 100 times higher than that of the *R*-isomer **2a**.

3.2 Structural effects on the insecticidal activity against American cockroaches

The α-phenethyl esters of the pyrazolinecarboxylic acid also showed insecticidal activity against the cockroach (Table 2). Without synergists, the activity values were close for all of the stereoisomers. The activity of isomers **8a** and **8b**, but not of **7a** and **7b**, increased by about twice under synergistic conditions with PB. By additional use of NIA, the activities of isomers **7a** and **7b** increased by about 10 times, whereas those of **8a** and **8b** increased by about five times. Thus, even though the effects of each metabolic inhibitor on the activity seemed to be somewhat different, the insecticidal activity values of the four isomers were similar under the synergistic conditions with PB and NIA.

Substitution of the *N*-(phenyl)carbamoyl and *N*-(4-chlorophenyl)carbamoyl moieties of compounds **1** and **2**, respectively, by corresponding arylacetyl groups (compounds **3** and **4**) or aryloxyacetyl groups (compounds **5** and **6**) gave compounds with very low activities which were practically inactive against house flies. Introduction of Cl at the *para* position increased the activity in compound **2**, but not in compounds **4** and **6**.

3.3 Conformational analysis of two selected pyrazolines

Profiles of the relative heats of formation as a function of rotation around the C(=O)—NH and C(=O)—CH_2 bonds for compound **2a** and the *R*-isomer of compound **4**, respectively, are given in Fig. 4. The minimum energy for compound **2a** was given for a conformer in which the N^1—C(=O) and NH—C_6H_4(4-Cl) bonds roughly take the *anti*-position. Rotation of the C(=O)—CH_2

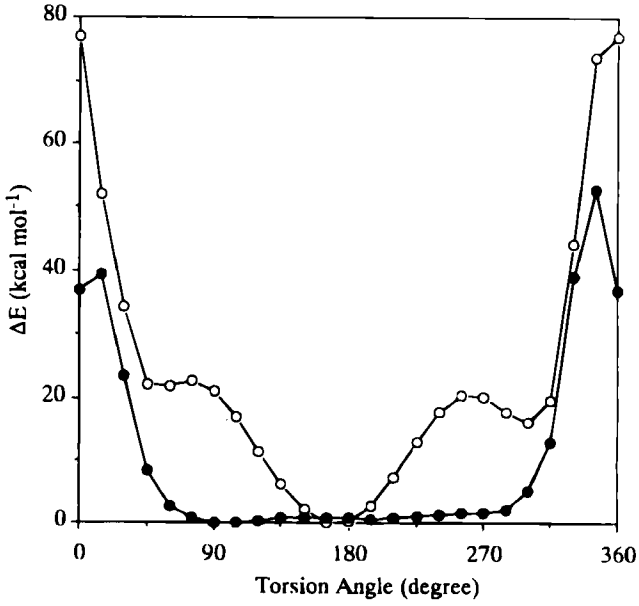


Fig. 4. Conformation–energy plots of (○) **2a** and (●) the *R*-isomer of **4**. Torsion angles of the C(=O)—NH bond for **2a** and of the C(=O)—CH_2 bond for the *R*-isomer of **4** were defined in the text. ΔE shows the energy for each conformer of each compound relative to that having the minimum energy among the conformers.

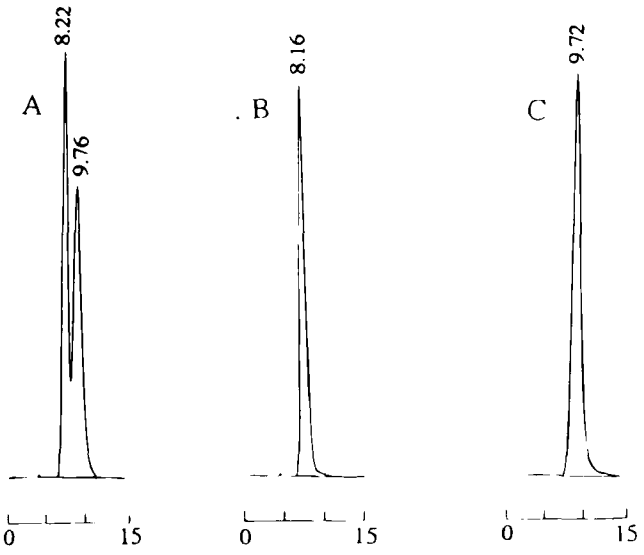


Fig. 5. HPLC chromatograms for (A) **2**, (B) **2a** and (C) **2b**. Conditions for analysis: column, Ceramosphere Ru-1, 4·6 × 250 mm Shiseido Co.; mobile phase, methanol; flow rate, 0·5 ml min^{-1}; detection, UV 254 nm.

bond of the *R*-isomer of compound **4** from the *anti*-position up to about 90° in either direction did not cause significant changes in the energy.

4 DISCUSSION

The HPLC analysis of racemic compound **2** on a chiral column gave two peaks at retention times of 8·22 and 9·76 min (Fig. 5). The LC–MS spectrum of compound **2** obtained by atmospheric pressure chemical ionization (at 2000 *amu*, *+ve* mode, Hitachi M1200H model) exhibited the molecular ion (M + 1) peak at *m/z* 406 for both peaks. These findings suggested that the two peaks correspond to the enantiomers of compound **2**. Conversion of the methyl ester moiety on the C-4 atom of the pyrazoline ring of compound **2** to the corresponding (*R*)- and (*S*)-α-phenethyl esters gave two separable pairs of diastereomers **7a** + **7b** and **8a** + **8b**, respectively, having a common molecular weight. In order to clarify the configuration at the C-4 atom of the pyrazoline ring, a single-crystal X-ray analysis was made for compound **8a**. Figure 6 shows that the C-4 atom of the pyrazoline ring possesses an *R*-configuration, while the C-1 atom in the side-chain alcohol moiety has an *S*-configuration. From the stereochemically defined compounds **8a** and **8b**, enantiomers **2a** and **2b** were obtained by transesterification with sodium methoxide. The retention time of each peak of compound **2** on the HPLC record agreed well with that of these enantiomers (Fig. 5).

The synergistic ratio in the insecticidal activity of compound **2a** was very close to that of compound **2b** (Table 1), indicating that the difference in the potency between the enantiomers is not due to that in the metabolic degradability. The ratio of the insecticidal activity against American cockroaches of the *S*-isomer **2b** to that of the *R*-isomer **2a** was about 10, whereas a similar ratio against house flies was about 100. When large amounts of weak compounds such as **2a** were topically applied to house flies, the compounds were often precipitated on the abdomen because of evaporation of methanol, resulting in a lower activity against house flies than would have been expected from the activity against American cockroaches. It is considered that the activity values against American cockroaches measured by injection reflect more precisely those at target sites.

When the methoxycarbonyl group of the more active isomer **2b** was substituted by (*R*)- and (*S*)-α-phenethyloxycarbonyl groups to give compounds **7b** and **8b**, respectively, the chiral effects on the insecticidal activity

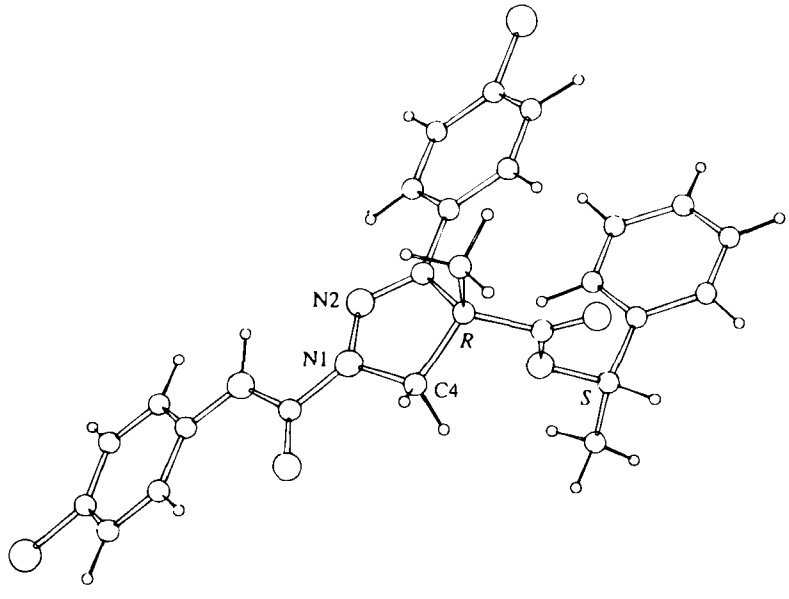


Fig. 6. Crystal structure of **8a**.

of the C-4 atom of the pyrazoline ring diminished (Table 2). Their activity was close to that of the less active enantiomer **2a** of compound **2**. The insecticidal activity of compound **2a** remained even after substitution of its methoxycarbonyl group by (*R*)- or (*S*)-α-phenethyloxycarbonyl group. These findings indicate that the insecticidal activity of this class of compounds is around log(1/MLD) = 8·5. When one of the substituents on the C-4 atom is methoxycarbonyl and the carbon atom has the *S*-configuration, another factor is likely to increase the activity. The α-phenethyloxy groups of compounds **7b** and **8b** may be too bulky to fit well with the receptor sites, so that the receptor sites could not recognize the chirality either of the alcohol moiety or of the C-4 atom of the pyrazoline ring.

Previously, it had been found that the greater the electron-withdrawing property of the *para* substituent on the benzene ring of the *N*-(phenyl)carbamoyl moiety of some compounds of structure **A** (A = CH_3, B = $COOCH_3$, Y = 4-Cl, Z = O), the higher the insecticidal activity.[12] It was suggested that the nitrogen atom of the NH group of the carbamoyl moiety had a specific role to give a conformation of the molecule suitable for the potential insecticidal effect. The X-ray data for compound **8a** provided additional information (Fig. 6). To examine this situation, the conformation–energy profiles for compound **2a** and the corresponding *N*-(4-chlorophenyl)acetyl derivative (*R*-isomer of compound **4**) were compared. The analysis revealed that the C(= O)-NH bond of compound **2a** mostly exists in the *anti*-position. This conformation may be suitable for the compound to interact with the receptor sites. The C(=O)—CH_2 bonds of compounds **3**, **5** and **6** may be too flexible to fit as well with the receptor sites as the bond of the *R*-isomer of compound **4**.

In summary, the stereochemistry of pyrazoline molecules was shown to be an important factor controlling their insecticidal effects. When the substituents at the C-4 atom of the pyrazoline ring were methyl and methoxycarbonyl, the chiral effects on the activity were clearly shown. By substituting the methoxycarbonyl group by (*R*)- or (*S*)-α-phenethyloxycarbonyl, the chiral effects at the C-4 atom diminished. The *anti*-position of the NH-aryl and N^1—C(=O) bonds is likely to give a conformation suitable for the insecticidal effects.

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